

Institut für Lebensmittelsicherheit und -hygiene  
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. Dr. h. c. Roger Stephan

Arbeit unter wissenschaftlicher Betreuung von  
Prof. Dr. Claudio Zweifel

**Process analysis of cattle slaughtering in two abattoirs: influence of  
process stages on the microbiological contamination of carcasses**

Inaugural-Dissertation

zur Erlangung der Doktorwürde der  
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

**Michel Capek**

Tierarzt  
von Adliswil, Zürich

genehmigt auf Antrag von  
Prof. Dr. Dr. h. c. Roger Stephan, Referent  
Prof. Dr. Michael Hässig, Korreferent

**2015**

## **Contents**

|           |  |           |
|-----------|--|-----------|
| <b>1.</b> | <b>Summary</b>   | <b>2</b>  |
| <b>2.</b> | <b>Introduction</b>  | <b>4</b>  |
| <b>3.</b> | <b>Material and Methods</b>  | <b>5</b>  |
| 3.1.      | Abattoirs and slaughter process  | 5         |
| 3.2.      | Sampling   | 6         |
| 3.3.      | Total viable counts (TVC) and <i>Enterobacteriaceae</i>                              | 7         |
| 3.4.      | Data analysis  | 7         |
| <b>4.</b> | <b>Results</b>   | <b>8</b>  |
| 4.1.      | TVC from cattle carcasses during the slaughter process                               | 8         |
| 4.2.      | <i>Enterobacteriaceae</i> results from cattle carcasses during the slaughter process | 10        |
| 4.3.      | Microbiological contamination of cattle hides and corresponding carcasses            | 11        |
| <b>5.</b> | <b>Discussion</b>  | <b>13</b> |
| <b>6.</b> | <b>Conclusions</b>   | <b>16</b> |
| <b>7.</b> | <b>References</b>  | <b>17</b> |
| <b>8.</b> | <b>Acknowledgements</b>  |           |
| <b>9.</b> | <b>Curriculum Vitae</b>  |           |

## 1. Summary

At selected stages of slaughter (skinning, evisceration, trimming, washing, blast chilling), 100 cattle carcasses from two abattoirs were examined for total viable counts (TVC) and *Enterobacteriaceae* by swabbing at the neck, brisket, flank and rump. After skinning, average TVC on carcasses was  $1.5 \log_{10}$  CFU cm<sup>-2</sup> and *Enterobacteriaceae* frequencies at sites were  $\leq 6\%$ . From skinned to washed carcasses, levels tended to slightly increase. Blasting clearly reduced microbiological results (TVC and *Enterobacteriaceae*) on carcasses from abattoir B, but reductions were limited or lacking in abattoir A. In addition, 100 hides and corresponding chilled carcasses were examined. On hides, average TVC was  $5.6 \log_{10}$  CFU cm<sup>-2</sup> and *Enterobacteriaceae* were commonly detected. Average carcass–hide ratios of the two abattoirs were comparable for TVC (abattoir A: 0.0182%; abattoir B: 0.0202%) but differed for *Enterobacteriaceae* counts (abattoir A: 0.4627%; abattoir B: 0.0941%). Such ratios allow comparing process performance between abattoirs in the daily practice.

**Keywords:** Cattle carcasses; slaughter process; cattle hides; carcass–hide ratio; total viable counts; *Enterobacteriaceae*

## Zusammenfassung

An ausgewählten Prozessstufen der Rinderschlachtung (nach Enthäuten, nach Evisceration, vor Abduschen, nach Abduschen, im Kühlraum) wurden 100 Rinderschlachttierkörper aus zwei Schlachtbetrieben beprobt. Die Proben wurden mittels Nass-Trockentupfertechnik an vier Entnahmestellen (Hals, Brust, Flanke, Keule) erhoben und auf die Gesamtkeimzahl (GKZ) und *Enterobacteriaceae* untersucht. Nach dem Enthäuten lag die mittlere GKZ der Schlachttierkörper bei  $1.5 \log_{10}$  KBE  $\text{cm}^{-2}$  und die *Enterobacteriaceae*-Nachweisraten der Entnahmestellen schwankten von 0% bis 6%. Im weiteren Verlauf der Schlachtung (bis nach Abduschen) stiegen die Werte leicht an. Im Kühlraum lagen nach der Schockkühlung im Betrieb B deutliche Reduktionen der GKZ- und *Enterobacteriaceae*-Ergebnisse vor, während im Betrieb A die Reduktionen geringer ausfielen oder fehlten. Zudem wurden 100 Rinderfelle und die zugehörigen, gekühlten Schlachttierkörper untersucht. Auf den Fellen lag die mittlere GKZ bei  $5.6 \log_{10}$  KBE  $\text{cm}^{-2}$  und *Enterobacteriaceae* wurden häufig gefunden. Die Berechnung der so genannten „carcass–hide ratios“ zeigte, dass für die GKZ die mittleren Werte in den beiden Betrieben vergleichbar waren (Betrieb A: 0.0182%; Betrieb B: 0.0202%), während sich bei den *Enterobacteriaceae* betriebsspezifische Unterschiede zeigten (Betrieb A: 0.4627%; Betrieb B: 0.0941%). Solche Werte erlauben Vergleiche der Prozesshygiene-Bedingungen verschiedener Betriebe in der täglichen Praxis.

**Schlüsselwörter:** Schlachtprozess; Rinder-Schlachttierkörper; Rinderfelle; carcass–hide ratios; Gesamtkeimzahl; *Enterobacteriaceae*

## 2. Introduction

To ensure food safety at slaughter, additional measures to the traditional meat inspection procedures are required, in particular because healthy food-producing animals can be carriers of important bacterial pathogens causing human illness (EFSA/ECDC, 2013; Nørrung and Buncic, 2008). Such pathogens might enter the food chain by direct or indirect fecal contamination, if good hygiene practices are not warranted. Strict adherence to good practices of slaughter hygiene, along with risk-based preventive measures, is therefore crucial to ensure both public health protection and meat quality. In the European Union (EU), food hygiene legislation (Reg. [EC] No. 853/2004 and 853/2004) places the focus on food business operators. They must apply compulsory self-checking programs following the hazard analysis and critical control points (HACCP) approach.

For assessment of process performance, analysis of the slaughter process is of central importance. To enable risks involved to be estimated and appropriate measure to be taken, slaughter process analysis must also include abattoir-specific microbiological data on carcass contamination during slaughter (Brown et al., 2000; Nørrung and Buncic, 2008; Spescha et al., 2006), especially because carcasses might be contaminated despite the absence of visible contamination (Gill, 2004). For verification of slaughter hygiene conditions in the daily practice, the microbial status of carcasses is often determined by monitoring indicator organisms on carcasses at the end of slaughter (Brown et al., 2000; Ruby et al., 2007; Zweifel et al., 2005). In the EU, Reg. (EC) No. 2073/2005 and 1441/2007 set out microbial criteria for carcasses at the end of slaughter. Because of the shortcomings of such end-point criteria, comparison of the microbial contamination on hides and corresponding carcasses has been proposed (Blagojevic et al., 2011; Vivas Alegre and Buncic, 2004).

The aims of this study were (i) to investigate the effects of certain cattle slaughter process stages on the microbial carcass contamination and (ii) to determine the quantitative relationship between the carcass and hide microflora in two large-scale Swiss abattoirs. Identification of abattoir-specific process stages increasing or decreasing microbial carcass contamination is required for the implementation of HACCP-based systems.

### 3. Material and Methods

#### 3.1. Abattoirs and slaughter process

This study was based on investigations carried out during seven months (December 2012 to June 2013) in two Swiss abattoirs with annual slaughter capacities of >20 million kg (abattoir A: cattle, sheep, pigs; abattoir B: only cattle). Abattoir A processed up to 60 cattle carcasses per h (on average 85 carcasses per day) and abattoir B up to 75 cattle carcasses per h (on average 450 carcasses per day). Slaughter operations were performed on mechanized slaughter lines featuring separated wet areas and clean areas (Table 1).

Table 1: Tasks performed in the cattle slaughter processes

| Location   | Process stages in abattoirs A and B  |
|------------|--|
| Wet Area   | Lairage  |
|            | Captive bolt stunning; shackling by right rear leg   |
|            | Sticking and bleeding <sup>b</sup>   |
|            | Removal of head and hooves   |
|            | Manual pre-skinning: skin incisions and pre-skinning of rear legs, rump, flank, tail, brisket and forelegs               |
| Clean Area | Skinning by upward-pulling hide puller <sup>a</sup>  |
|            | Evisceration: brisket sawing, freeing of bung, removal of gut and thoracic viscera <sup>a</sup>                          |
|            | Carcass splitting with a saw (use of cold water)   |
|            | Meat inspection and stamping   |
|            | Trimming: trimming of butt, rump and brisket; removal of mesenteric fat, diaphragm remnants and spinal cord <sup>a</sup> |
|            | Carcass weighing and grading   |
| Chiller    | Final cold water washing <sup>a</sup>  |
|            | Two-stage air chilling process: conventional chilling with preceding blasting <sup>a,b</sup>                             |

<sup>a</sup> Process stages surveyed for bacterial counts in the slaughter process analysis

<sup>b</sup> Process stages surveyed for bacterial counts in order to determine carcass–hide ratios

After being stunned using a captive bolt, animals were shackled by the right rear leg and immediately exsanguinated. Before skinning, head and hooves were removed. Skinning comprised manually performed pre-skinning and mechanized skinning by an upward-pulling hide puller. Before evisceration, carcasses were moved into separated clean areas. Evisceration involved slitting the belly, removal of the gut and removal of thoracic viscera. Carcasses were then split along the midline from back to front with a splitting saw. After trimming, meat inspection, weighing and grading, carcasses were washed with cold potable water to remove visual debris (abattoir A: 12 °C for 16 s; abattoir B: 11 °C for 20 s). Both abattoirs used a two-stage air chilling process. At abattoir A, carcasses were initially blasted with air at 16 m/s and -8.0 °C for about 45 min before entering the chiller (6 m/s at 0–2.0 °C). At abattoir B, air speed and temperature were 11 m/s and 10 °C during blasting (90 min) and 5 m/s and 2.0–4.0 °C in the chiller.

### **3.2. Sampling**

Sampled animals were aged between three and 24 months and their origin was distributed throughout Switzerland. Sampling comprised two parts in both abattoirs. First, cattle carcasses were sampled after selected stages of slaughter (skinning, evisceration, trimming, washing, blast chilling). At each stage and abattoir, 50 carcasses were examined. Second, 100 cattle hides and corresponding carcasses were sampled ( $n = 50$  at each abattoir). Samples were collected from hides after sticking and from carcass in the chiller after blasting. Carcass and hide samples were obtained from the neck, brisket, flank and rump area using the wet-dry double swab technique. At each site, first a moistened swab (0.85% saline solution) and then a dry swab was rubbed across the sampling site (100 cm<sup>2</sup>). Samples were transported to the laboratory chilled and microbiological examinations were carried out within 5 h after sampling.

### 3.3. Total viable counts (TVC) and *Enterobacteriaceae*

Both swabs of each sampling site were homogenized for 60 s in 20 ml of 0.85% saline solution in a stomacher. Suspensions were plated with a spiral plater (Eddy Jet, IUL SA, Barcelona, Spain) onto plate count agar (Oxoid AG, Pratteln, Switzerland) for TVC and violet red bile glucose agar (VRBG agar; BBL, Cockeysville, MD, USA) for *Enterobacteriaceae*. Plate count agar was incubated aerobically for 72 h at 30 °C and VRBG agar was incubated anaerobically for 48 h at 30 °C. Counts were calculated as CFU cm<sup>-2</sup> and the detection limit was 4 CFU cm<sup>-2</sup> for carcass samples.

### 3.4. Data analysis

Counts were expressed as log<sub>10</sub> CFU cm<sup>-2</sup> and compared by reference to mean log<sub>10</sub> ( $\bar{x}$ ) values. Evaluation was based on log<sub>10</sub> *N* (log<sub>10</sub> of summed counts) when the occurrence was too infrequent (<80%) to ensure log<sub>10</sub> normality (McEvoy et al., 2004). Values differing by <0.5 ( $\bar{x}$ ) or <1.0 log<sub>10</sub> CFU cm<sup>-2</sup> (log<sub>10</sub> *N*) were regarded as similar for practical purposes. For *Enterobacteriaceae*, frequencies were additionally determined. Statistical analysis was performed using IBM SPSS Statistics 20 (IBM, Armonk, NY, USA). Analysis of variance and the Bonferroni procedure were used to analyze differences in bacterial counts between process stages, sampling sites and abattoirs. Contingency tables (Chi square test, Fisher exact test) were used to compare *Enterobacteriaceae* frequencies. In addition, carcass–hide ratios were calculated (Blagojevic et al., 2011): carcass–hide ratio (%) =  $\frac{\sqrt[n]{x_1 * x_2 * \dots * x_n} (carcass)}{\sqrt[n]{x_1 * x_2 * \dots * x_n} (hide)} * 100$ , where *x* is CFU cm<sup>-2</sup> (*x* = 0 for results below detection limit).



## 4. Results

### 4.1. TVC from cattle carcasses during the slaughter process

After skinning, mean  $\log_{10}$  TVC at the different sampling sites ranged from 1.1 to 2.8  $\log_{10}$  CFU  $\text{cm}^{-2}$  in abattoir A and 0.9 to 1.7  $\log_{10}$  CFU  $\text{cm}^{-2}$  in abattoir B (Table 2, Fig. 1). At abattoir A, mean  $\log_{10}$  TVC from the neck and rump were similar but differed by about 0.5 and 1.7  $\log_{10}$  CFU  $\text{cm}^{-2}$  from the values of the flank ( $P < 0.05$ ) and brisket ( $P < 0.05$ ), respectively. The brisket thereby yielded clearly higher results than (i) the other sites also on later stages (evisceration, trimming, washing) ( $P < 0.05$ ) and (ii) the brisket of carcasses from abattoir B ( $P < 0.05$ ). At abattoir B, TVC from the rump of skinned carcasses differed significantly from the values of the other sites ( $P < 0.05$ ).

Table 2: TVC results ( $\log_{10}$  CFU  $\text{cm}^{-2}$ ) from cattle carcasses at selected stages of slaughter (n = 50 at each site, process stage and abattoir)<sup>a</sup>

| Abattoir | Process stage         | Neck      |      | Brisket   |      | Flank     |      | Rump      |      |
|----------|-----------------------|-----------|------|-----------|------|-----------|------|-----------|------|
|          |                       | $\bar{x}$ | SD   | $\bar{x}$ | SD   | $\bar{x}$ | SD   | $\bar{x}$ | SD   |
| A        | After skinning        | 1.08      | 0.88 | 2.80      | 0.84 | 1.63      | 1.05 | 1.11      | 1.05 |
|          | After evisceration    | 1.03      | 0.90 | 2.60      | 0.69 | 2.08      | 1.03 | 1.05      | 0.95 |
|          | After trimming        | 1.88*     | 0.94 | 2.86      | 0.68 | 1.41*     | 1.03 | 1.58*     | 0.83 |
|          | After washing         | 1.76      | 0.62 | 2.65      | 0.71 | 1.76      | 0.73 | 1.96      | 0.67 |
|          | Chilling <sup>b</sup> | 2.25*     | 0.71 | 2.24      | 0.70 | 1.52      | 0.71 | 1.90      | 0.88 |
| B        | After skinning        | 1.38      | 0.72 | 1.73      | 0.88 | 1.57      | 0.62 | 0.94      | 0.76 |
|          | After evisceration    | 1.23      | 0.69 | 1.61      | 0.83 | 1.47      | 0.79 | 0.75      | 0.67 |
|          | After trimming        | 1.45      | 0.64 | 1.69      | 0.69 | 1.42      | 0.76 | 1.10      | 0.76 |
|          | After washing         | 1.58      | 0.64 | 2.17*     | 0.75 | 1.55      | 0.73 | 1.50*     | 0.62 |
|          | Chilling <sup>b</sup> | 1.07*     | 0.72 | 1.32*     | 0.78 | 0.92*     | 0.72 | 0.84*     | 0.56 |

<sup>a</sup>  $\bar{x}$ , mean  $\log_{10}$  CFU  $\text{cm}^{-2}$ ; SD, standard deviation

<sup>b</sup> In the chiller after blasting

\* Significant change compared to the preceding process stage

Evisceration did not cause significant changes of TVC (Table 2, Fig. 1). Differences to the corresponding mean  $\log_{10}$  TVC after skinning were mainly  $\leq 0.2 \log_{10}$  CFU  $\text{cm}^{-2}$ . After trimming, TVC increases (neck, brisket, rump) and decreases (flank) were observed, but only changes at the neck, flank and rump in abattoir A were  $>0.5 \log_{10}$  CFU  $\text{cm}^{-2}$  ( $P<0.05$ ). Similarly, washing resulted in increases or decreases. Resulting mean  $\log_{10}$  TVC from the neck, flank and rump were comparable (abattoir A: 1.8–2.0  $\log_{10}$  CFU  $\text{cm}^{-2}$ ; abattoir B: 1.5–1.6  $\log_{10}$  CFU  $\text{cm}^{-2}$ ), whereas higher values were found for the brisket ( $P<0.05$ ). Blasting mainly reduced TVC, especially in abattoir B (Table 2, Fig. 1). At abattoir B, reductions of mean  $\log_{10}$  TVC at sites ranged from 0.5 to 0.9  $\log_{10}$  CFU  $\text{cm}^{-2}$  ( $P<0.05$ ), whereas reductions were lower or lacking in abattoir A. Resulting TVC differed significantly between the two abattoirs ( $P<0.05$ ) and mean  $\log_{10}$  TVC ranged from 1.5 to 2.3  $\log_{10}$  CFU  $\text{cm}^{-2}$  in abattoir A (forequarter  $>$  flank,  $P<0.05$ ) and 0.8 to 1.3  $\log_{10}$  CFU  $\text{cm}^{-2}$  in abattoir B (brisket  $>$  hindquarter,  $P<0.05$ ).

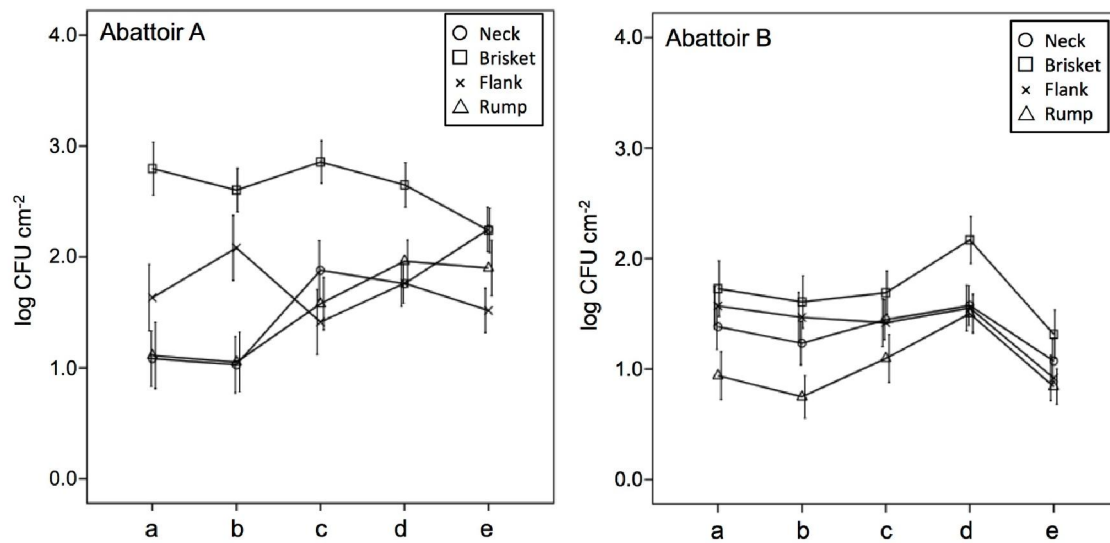


Figure 1: TVC results (mean  $\log_{10}$  CFU  $\text{cm}^{-2}$ ) on cattle carcasses at the neck (O), brisket (□), flank (x) and rump (Δ) after (a) skinning, (b) evisceration, (c) trimming, (d) washing and (e) blast chilling (n = 50 at each site, stage and abattoir; error bars represent 95% confidence intervals)

#### 4.2. *Enterobacteriaceae* results from cattle carcasses during the slaughter process

*Enterobacteriaceae* frequencies and  $\log_{10} N$  values are shown in Table 3. Counts of the 93 *Enterobacteriaceae*-positive samples were mainly (82.8%)  $<1.0 \log_{10} \text{CFU cm}^{-2}$ . Only two (2.4%) samples exceeded  $2.0 \log_{10} \text{CFU cm}^{-2}$ . At sequential stages of slaughter, only few changes of *Enterobacteriaceae* frequencies were significant (Table 3). At abattoir A, frequencies after skinning, washing and blasting ranged from 0 to 6%, 2 to 8% and 6 to 20%, respectively. Amongst sites, the brisket tended to yield slightly higher frequencies (up to blasting). Significant differences ( $P<0.05$ ) were evident after evisceration (brisket/flank versus neck/rump), after trimming (brisket versus flank) and in the chiller (rump versus other sites). At abattoir B, frequencies ranged from 0 to 2% after skinning and 4 to 14% after washing, whilst *Enterobacteriaceae* were not detected after blasting. Amongst sites, significant differences ( $P<0.05$ ) were only evident after trimming (neck versus other sites).

Table 3: *Enterobacteriaceae* results from cattle carcasses at selected stages of slaughter (n = 50 at each site, process stage and abattoir)<sup>a</sup>.

| Abattoir | Process stage         | Neck  |          | Brisket |          | Flank |                 | Rump  |          |
|----------|-----------------------|-------|----------|---------|----------|-------|-----------------|-------|----------|
|          |                       | % pos | $\log N$ | % pos   | $\log N$ | % pos | $\log N$        | % pos | $\log N$ |
| A        | After skinning        | 4     | 0.90     | 6       | 1.08     | 0     | ND <sup>b</sup> | 0     | ND       |
|          | After evisceration    | 0     | ND       | 10      | 2.58     | 10    | 1.56            | 0     | ND       |
|          | After trimming        | 4     | 1.45     | 10      | 2.39     | 0     | ND              | 2     | 1.08     |
|          | After washing         | 2     | 0.60     | 8       | 1.30     | 2     | 0.60            | 2     | 0.90     |
|          | Chilling <sup>c</sup> | 6     | 1.75     | 6       | 1.30     | 6     | 1.30            | 20*   | 2.13     |
| B        | After skinning        | 2     | 0.90     | 2       | 0.60     | 0     | ND              | 2     | 0.60     |
|          | After evisceration    | 0     | ND       | 6       | 1.08     | 2     | 0.60            | 4     | 1.08     |
|          | After trimming        | 16*   | 1.51     | 8       | 1.38     | 2     | 1.08            | 4     | 0.90     |
|          | After washing         | 10    | 1.51     | 14      | 1.56     | 4     | 0.90            | 12    | 1.51     |
|          | Chilling <sup>c</sup> | 0     | ND       | 0*      | ND       | 0     | ND              | 0*    | ND       |

<sup>a</sup> % pos, percentage of *Enterobacteriaceae*-positive samples;  $\log N$ ,  $\log_{10}$  of the total number recovered per square centimeter

<sup>b</sup> ND, no data for calculation (*Enterobacteriaceae* not detected); <sup>c</sup> In the chiller after blasting

\* Significant change (frequency) compared to the preceding process stage

### 4.3. Microbiological contamination of cattle hides and corresponding carcasses

On hides from abattoir A, mean  $\log_{10}$  TVC, mean  $\log_{10}$  *Enterobacteriaceae* counts and *Enterobacteriaceae* frequencies at sites ranged from 5.7 to 6.1  $\log_{10}$  CFU  $\text{cm}^{-2}$ , 1.2 to 1.7  $\log_{10}$  CFU  $\text{cm}^{-2}$  and 76 to 96%, respectively (Table 4).

Table 4: TVC and *Enterobacteriaceae* results ( $\log_{10}$  CFU  $\text{cm}^{-2}$ ) from cattle hides (n = 50 at each site and abattoir)<sup>a</sup>

| Abattoir | Site    | TVC       |      |      | <i>Enterobacteriaceae</i> |      |      |       |
|----------|---------|-----------|------|------|---------------------------|------|------|-------|
|          |         | $\bar{x}$ | SD   | Max  | $\bar{x}$                 | SD   | Max  | % pos |
| A        | Neck    | 5.69      | 0.64 | 7.14 | 1.34                      | 0.90 | 3.12 | 84    |
|          | Brisket | 5.69      | 0.66 | 7.14 | 1.22                      | 0.92 | 3.01 | 76    |
|          | Flank   | 6.06      | 0.71 | 7.38 | 1.65                      | 0.73 | 3.30 | 96    |
|          | Rump    | 5.92      | 1.07 | 7.11 | 1.49                      | 0.96 | 3.15 | 80    |
| B        | Neck    | 4.97      | 1.15 | 6.94 | 1.38                      | 1.09 | 3.52 | 74    |
|          | Brisket | 5.63      | 0.55 | 6.82 | 1.30                      | 0.91 | 2.86 | 78    |
|          | Flank   | 5.50      | 0.96 | 7.00 | 1.72                      | 1.01 | 3.43 | 88    |
|          | Rump    | 5.18      | 1.18 | 6.33 | 1.45                      | 1.07 | 3.28 | 78    |

<sup>a</sup>  $\bar{x}$ , mean  $\log_{10}$  CFU  $\text{cm}^{-2}$ ; SD, standard deviation; Max, maximum ( $\log_{10}$  CFU  $\text{cm}^{-2}$ ); % pos, percentage of *Enterobacteriaceae*-positive samples

Respective values on hides from abattoir B ranged from 5.0 to 5.6  $\log_{10}$  CFU  $\text{cm}^{-2}$ , 1.3 to 1.7  $\log_{10}$  CFU  $\text{cm}^{-2}$  and 74 to 88%, respectively. Hides from abattoir A tended to yield higher TVC than those from abattoir B ( $P < 0.05$ ), whilst *Enterobacteriaceae* results were comparable. Amongst sites, the flank and the brisket (TVC in abattoir B) tended to yield higher results, but only selected differences were significant (TVC: neck versus brisket/flank in abattoir B; *Enterobacteriaceae* frequencies: flank versus other sites in abattoir A;  $P < 0.05$ ). TVC between hides and corresponding carcasses differed on average by 3.7 and 4.1  $\log_{10}$  CFU  $\text{cm}^{-2}$  in abattoir A and B, respectively (Fig. 2).

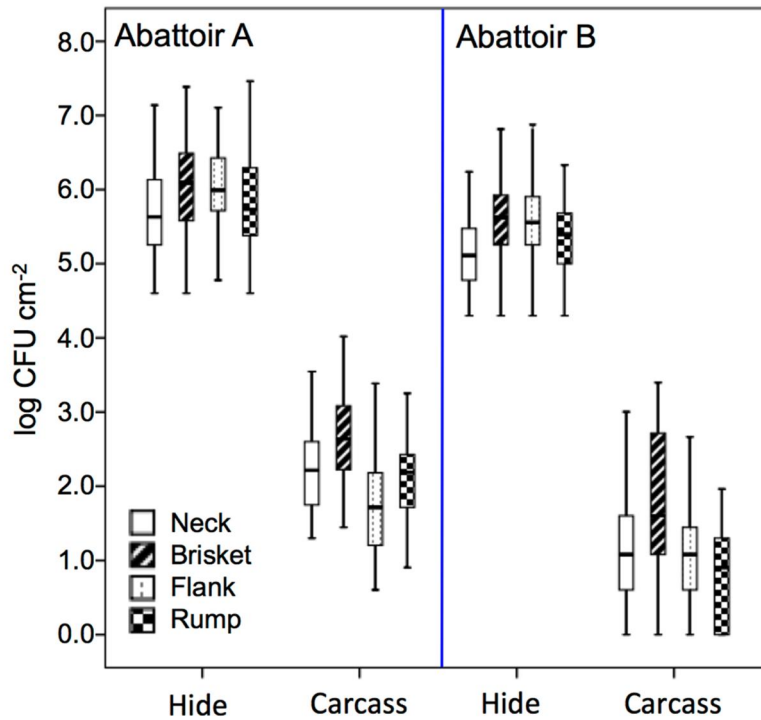


Figure 2: TVC results ( $\log_{10}$  CFU  $\text{cm}^{-2}$ ) from cattle hides and corresponding carcasses in the chiller after blasting ( $n = 50$  at each site and abattoir)

Calculation of carcass–hide ratios showed that average TVC on cattle carcasses was 0.0182% of hide TVC in abattoir A (0.0085–0.0285% at sites) and 0.0202% in abattoir B (0.0035–0.0343% at sites) (Table 5). Carcass–hide ratios from forequarters were comparable in the two abattoirs and tended to be higher than those from hindquarters. Factors from the flank and rump differed 1.6-fold and 2.5-fold between the abattoirs, respectively. For *Enterobacteriaceae* counts, carcass–hide ratios at sites were between 3.7-fold and 6.9-fold higher in abattoir A (0.2581–0.5872%) than in abattoir B (0.0663–0.1154%).

Table 5: Quantitative relationship between carcass and hide microflora of slaughtered cattle (hides sampled after sticking, corresponding carcasses sampled in the chiller after blasting; n = 50 at each site and abattoir)

| Microorganisms                   | Abattoir | Carcass–hide ratio (%) <sup>a</sup> |         |        |        |
|----------------------------------|----------|-------------------------------------|---------|--------|--------|
|                                  |          | Neck                                | Brisket | Flank  | Rump   |
| TVC                              | A        | 0.0270                              | 0.0285  | 0.0085 | 0.0087 |
|                                  | B        | 0.0295                              | 0.0343  | 0.0134 | 0.0035 |
| <i>Enterobacteriaceae</i> counts | A        | 0.4321                              | 0.5872  | 0.2581 | 0.5735 |
|                                  | B        | 0.1154                              | 0.0857  | 0.0663 | 0.1090 |

<sup>a</sup> Calculation in accordance with Blagojevic et al. (2011)

## 5. Discussion

Cattle hides are considered the primary source of carcass contamination during slaughter. In particular during skinning operations, bacteria including important foodborne pathogens may be transferred from hides onto carcasses via direct and indirect contacts. In the present study, average TVC on hides were 5.8 log<sub>10</sub> CFU cm<sup>-2</sup> in abattoir A (5.7–6.1 log<sub>10</sub> CFU cm<sup>-2</sup> at sites) and 5.3 log<sub>10</sub> CFU cm<sup>-2</sup> in abattoir B (5.0–5.6 log<sub>10</sub> CFU cm<sup>-2</sup> at sites). *Enterobacteriaceae*, which are used as indicators of fecal contamination (and thereby a parameter for bacterial pathogens), were frequently detected at the different sites (74–96%). Comparable and higher microbial loads are commonly found on cattle hides (Arthur et al., 2004; Bacon et al., 2000; Bell, 1997; Brichta-Harhay et al., 2008; Serraino et al., 2012). Most contaminated hide areas might vary, but certain areas as the brisket and flank tend to be more contaminated than others (Antic et al., 2010; Gill, 2004; Reid et al., 2002). To reduce the microbial contamination, various hide decontamination treatments have been proposed, but data obtained under commercial conditions are limited (Loretz et al., 2011).

For identification of abattoir-specific hygienic weak points, slaughter process analysis including microbiological data is required. This is of special interest in Europe because current legislation only permits the use of potable water (Reg. [EC] No. 853/2004) and lactic acid (Reg. [EC] No. 101/2013) to reduce the microbial contamination on cattle carcasses. Besides, the application of various decontamination treatments as typically used in North America hampers the comparability of results from different studies (Loretz et al., 2011).

Microbiological process analysis in the two abattoirs showed that microbial contamination of carcasses was generally low after skinning, in particular for carcasses not subjected to decontamination treatments. Average TVC after skinning were  $1.7 \log_{10}$  CFU  $\text{cm}^{-2}$  in abattoir A and  $1.4 \log_{10}$  CFU  $\text{cm}^{-2}$  in abattoir B, whilst *Enterobacteriaceae* frequencies at sites were comparable and ranged from 0 to 6%. However, an exception was the increased microbial load at the brisket of carcasses from abattoir A (mean  $\log_{10}$  TVC:  $2.8 \log_{10}$  CFU  $\text{cm}^{-2}$ ), which might be related to skin-opening cuts and hide-meat contacts in this area (Bell, 1997; Hauge et al., 2012; McEvoy et al., 2004). Published data indicate that microbial loads of skinned carcasses can vary widely (Arthur et al., 2004; Bacon et al., 2000; Brichta-Harhay et al., 2008; McEvoy et al., 2004; Ruby et al., 2007). Studies from Canada also reported low TVC (Gill et al., 2003; Yang et al., 2012), but hide washes with chemical compounds were used in the study of Yang et al. (2012).

At the following examined stages of slaughter (evisceration, trimming, washing), minor changes of TVC and *Enterobacteriaceae* results occurred, but comparison of results from skinned and washed carcasses mainly showed increases. Thus, eviscerating and trimming operations were performed without extensive additional contamination and washing with cold water was, as expected, not effective for reducing microbial loads and yielded rather redistributions (Bell, 1997; Loretz et al., 2011; McEvoy et al., 2004; Yang et al., 2012). However, certain abattoir- and site-specific effects were evident. For example, increased TVC at the neck after trimming (abattoir A) might be related to the water applied during carcass sawing and draining across the neck. Moreover, certain *Enterobacteriaceae* results provided indications of hygienic weak points at specific stages and sites not or not as clearly apparent from TVC data.

Only minor or site-specific changes of microbial loads during slaughter on carcasses not subjected to decontamination treatments have previously been reported (Madden et al., 2004; McEvoy et al., 2004). However, they found a less consistent situation for indicators of fecal contamination (McEvoy et al., 2004). In the present study, average TVC on carcasses before chilling were  $2.0 \log_{10}$  CFU  $\text{cm}^{-2}$  in abattoir A and  $1.7 \log_{10}$  CFU  $\text{cm}^{-2}$  in abattoir B, whilst *Enterobacteriaceae* frequencies at sites ranged from 2 to 14%.

Blast chilling clearly reduced TVC and *Enterobacteriaceae* results on carcasses from abattoir B, but reductions were limited or lacking in abattoir A. Published data indicate that chilling of cattle carcasses can result in increases, decreases or no changes of the microbial contamination, dependent on chilling method, temperature, air speed, humidity, duration, carcass spacing and carcass site (Arthur et al., 2004; Gill et al., 2003; McEvoy et al., 2004; Ruby et al., 2007; Yang et al., 2012). Reductions obtained by air chilling are mainly based on surface desiccation, but extreme regimes may cause quality problems (Loretz et al., 2011; Savell et al., 2005). The abattoir-specific effects of blasting in the present study were probably caused by the varying parameters used and the resulting differences in the achieved surface desiccation (only carcasses from abattoir B were visually dry afterwards). Resulting average TVC on carcasses were  $2.0 \log_{10}$  CFU  $\text{cm}^{-2}$  in abattoir A and  $1.0 \log_{10}$  CFU  $\text{cm}^{-2}$  in abattoir B. *Enterobacteriaceae* were found in varying frequencies in abattoir A but were not detected in abattoir B. In comparable studies (no use of decontamination treatments), average TVC of chilled cattle carcasses ranged from 2.1 to  $3.1 \log_{10}$  CFU  $\text{cm}^{-2}$  at different plants Switzerland (Zweifel et al., 2005), 2.4 to  $3.2 \log_{10}$  CFU  $\text{cm}^{-2}$  at different plants in Northern Ireland (Murray et al., 2001) and 2.5 to  $3.1 \log_{10}$  CFU  $\text{cm}^{-2}$  at different sampling sites in Ireland (McEvoy et al., 2004). Similar results were also reported in a Belgian study for carcasses sampled in the chiller 2 to 4 h after slaughter (Ghafir et al., 2008).

In addition, the quantitative relationship between carcass and hide microflora was determined (carcass–hide ratio). This approach includes information on the microbial status of the slaughtered animals and corresponding carcasses and thus the slaughter process performance (from skinning to the carcass sampling point).



In the present study, average carcass–hide ratios were comparable in the two abattoirs for TVC (abattoir A: 0.0182%; abattoir B: 0.0202%), albeit some site-specific differences were identified (forequarter > hindquarter). On the other hand, ratios for *Enterobacteriaceae* counts were higher in abattoir A (average: 0.4627%) than in abattoir B (average: 0.0941%). Taking into account the results from the microbiological process analysis, these differences are mainly caused by the effect of blasting. Only a few studies have determined carcass–hide ratios under commercial conditions (Blagojevic et al., 2011; Vivas Alegre and Buncic, 2004). Investigating two abattoirs, Blagojevic et al. (2011) reported ratios of 0.0116% and 0.0017% for TVC and 2.00% and 5.39% for *Enterobacteriaceae* but carcasses were sampled before chilling. Other recent studies addressed the hide–carcass transfer by using a contact model (Antic et al., 2010) or by comparing microbiological results from hides and pre- and post-intervention carcasses (Brichta-Harhay et al., 2008). Although more studies including abattoirs with varying capacities and technologies are required, determination of carcass–hide ratios as shown in this study provides information on the slaughter process performance and allows reliable comparisons between abattoirs.

## 6. Conclusions

Consequently, there was a considerable microbial contamination pressure (TVC and *Enterobacteriaceae*) associated with hides of cattle delivered for slaughter. Analysis of the microbial carcass contamination at selected stages of the cattle slaughter process identified certain abattoir- and site-specific differences. Results (TVC and *Enterobacteriaceae*) after skinning were generally low compared to published data. Minor changes occurred at the following stages (evisceration, trimming, washing), albeit comparison of results from skinned and washed carcasses mainly showed increases. Striking were the abattoir-specific effects observed in the chiller after blasting, which were probably related to differences in achieved surface desiccation. Furthermore, determination of the quantitative relationship between carcass and hide microflora allows comparing slaughter process performance between abattoirs in the daily practice.

## 7. References

- Antic D, Blagojevic B, Ducic M, Nastasijevic I, Mitrovic R, Buncic S (2010): Distribution of microflora on cattle hides and its transmission to meat via direct contact. *Food Control*, 21, 1025–1029.
- Arthur TM, Bosilevac JM, Nou X, Shackelford SD, Wheeler TL, Kent MP, Jaroni D, Pauling B, Allen DM, Koohmaraie M (2004): *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at various steps in commercial beef processing plants. *Journal of Food Protection*, 67, 658–665.
- Bacon RT, Belk KE, Sofos JN, Clayton RP, Reagan JO, Smith GC (2000): Microbial populations on animal hides and beef carcasses at different stages of slaughter in plants employing multiple-sequential interventions for decontamination. *Journal of Food Protection*, 63, 1080–1086.
- Bell RG (1997): Distribution and sources of microbial contamination on beef carcasses. *Journal of Applied Microbiology*, 82, 292–300.
- Blagojevic B, Antic D, Ducic M, Buncic S (2011): Ratio between carcass- and skin-microflora as an abattoir process hygiene indicator. *Food Control*, 22, 186–190.
- Brichta-Harhay DM, Guerini MN, Arthur TM, Bosilevac JM, Kalchayanand N, Shackelford SD, Wheeler TL, Koohmaraie M (2008): *Salmonella* and *Escherichia coli* O157:H7 contamination on hides and carcasses of cull cattle presented for slaughter in the United States: an evaluation of prevalence and bacterial loads by immunomagnetic separation and direct plating methods. *Applied and Environmental Microbiology*, 74, 6289–6297.
- Brown, MH, Gill CO, Hollingsworth J, Nickelson II R, Seward S, Sheridan JJ, Stevenson T, Sumner JL, Theno DM, Usborne WR, Zink D (2000): The role of microbiological testing in systems for assuring the safety of beef. *International Journal of Food Microbiology*, 62, 7–16.
- EFSA/ECDC, European Food Safety Authority/European Center for Disease Prevention and Control (2013): The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011. *EFSA Journal*, 3129.

- Ghafir Y, China B, Dierick K, De Zutter L, Daube G (2008): Hygiene indicator microorganism for selected pathogens on beef, pork, and poultry meats in Belgium. *Journal of Food Protection*, 71, 35–45.
- Gill CO (2004): Visible contamination on animals and carcasses and the microbiological condition of meat. *Journal of Food Protection*, 67, 413–419.
- Gill CO, Bryant J, Landers C (2003): Identification of critical control points for control of microbiological contamination in processes leading to the production of ground beef at a packing plant. *Food Microbiology*, 20, 641–650.
- Hauge SJ, Nafstad O, Røtterud O-J, Nesbakken T (2012): The hygienic impact of categorisation of cattle by hide cleanliness in the abattoir. *Food Control*, 27, 100–107.
- Loretz M, Stephan R, Zweifel C (2011): Antibacterial activity of decontamination treatments for cattle hides and beef carcasses. *Food Control*, 22, 347–359.
- Madden RH, Murray KA, Gilmour A (2004): Determination of the principal points of product contamination during beef carcass dressing processes in Northern Ireland. *Journal of Food Protection*, 63, 1494–1496.
- McEvoy JM, Sheridan JJ, Blair IS, McDowell DA (2004): Microbial contamination on beef in relation to hygiene assessment based on criteria used in EU Decision 2001/471/EC. *International Journal of Food Microbiology*, 92, 217–225.
- Murray KA, Gilmour A, Madden RH (2001): Microbiological quality of chilled beef carcasses in Northern Ireland: a baseline survey. *Journal of Food Protection*, 64, 498–502.
- Nørnung B, Buncic S (2008): Microbial safety of meat in the European Union. *Meat Science*, 78, 14–24.
- Reid C-A, Small A, Avery SM, Buncic S (2002): Presence of food-borne pathogens on cattle hides. *Food Control*, 13, 411–415.
- Ruby JR, Zhu J, Ingham SC (2007): Using indicator bacteria and *Salmonella* test results from three large-scale beef abattoirs over an 18-month period to evaluate intervention system efficacy and plan carcass testing for *Salmonella*. *Journal of Food Protection*, 70, 2732–2740.
- Savell JW, Mueller SL, Baird BE (2005): The chilling of carcasses. *Meat Science*, 70, 449–459.

- Serraino A, Bardasi L, Riu R, Pizzamiglio V, Liuzzo G, Galletti G, Giacometti F, Merialdi, G (2012): Visual evaluation of cattle cleanliness and correlation to carcass microbial contamination during slaughtering. *Meat Science*, 90, 502–506.
- Spescha C, Stephan R, Zweifel C (2006): Microbiological contamination of pig carcasses at different stages of slaughter in two European Union-approved abattoirs. *Journal of Food Protection*, 69, 2568–2575.
- Vivas Alegre L, Buncic S (2004): Potential use of hide-carcass microbial counts relationship as an indicator of process hygiene performance of cattle abattoirs. *Food Protection Trends*, 24, 814–820.
- Yang X, Badoni M, Youssef MK, Gill CO (2012): Enhanced control of microbiological contamination of product at a large beef packing plant. *Journal of Food Protection*, 75, 144–149.
- Zweifel C, Baltzer D, Stephan R (2005): Microbiological contamination of cattle and pig carcasses at five abattoirs determined by swab sampling in accordance with EU Decision 2001/471/EC. *Meat Science*, 69, 559–566.

## **8. Acknowledgements**

I would like to express my gratitude to all those who contributed to this work. Special thanks go to the staff of the slaughterhouses involved in this study for facilitating access to their operations and for assistance with the collection of data. This project was partially funded by the Swiss Army.

I owe my gratitude to

Prof. Dr. Roger Stephan, Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, for giving me the opportunity to work on this scientific project, for his optimistic support and help when ever needed.

Prof. Dr. Michael Hässig, Section of Herd Health, Farm Animal Department, Vetsuisse Faculty University of Zurich, for accepting to referee my dissertation.

Prof. Dr. Claudio Zweifel, Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, for his patience, his help whenever needed and the preparation of the main review.

The whole ILS team for their assistance and creation of a friendly working atmosphere.

My parents for their care and appreciation.

## 9. Curriculum Vitae

|                 |  |
|-----------------|--|
| Vorname Name    | Michel Capek   |
| Geburtsdatum    | 12. Juli 1985  |
| Geburtsort      | Zürich   |
| Nationalität    | Schweizer  |
| Heimatort       | Adliswil, ZH   |
| 08/1992-06/1995 | Primarschule Vilacker, Adliswil, Schweiz   |
| 08/1995-06/1997 | Mittelschule Gemeindeschulhaus, Kilchberg, Schweiz   |
| 08/1997-06/1999 | Orientierungsschule Burgstrasse, Basel, Schweiz  |
| 08/1999-07/2005 | Gymnasium Bäumlhof, Basel, Schweiz   |
| 25/06/2005      | Erlangung der Maturität am Gymnasium Bäumlhof, Basel, Schweiz; Schwerpunkt Biologie & Chemie   |
| 09/2005-08/2011 | Studium der Veterinärmedizin an der Vetsuisse-Fakultät Universität Zürich, Zürich, Schweiz   |
| 12/2012-09/2015 | Anfertigung der Dissertation unter Leitung von Prof. Dr. Claudio Zweifel am Institut für Lebensmittelsicherheit und -hygiene der Vetsuisse-Fakultät Universität Zürich, Zürich, Schweiz<br>Direktor: Prof. Dr. Dr. h. c. Roger Stephan |